WS7.1 Multi-target corrective effect of vardenafil on F508del-CFTR function and localization

B. Dhooghe[1], S. Noël[1], C. Bouzin[2], P. Lebecque[3], P. Wallemacq[1], T. Leal[1]. [1] *Université Catholique de Louvain, Louvain Centre for Toxicology and Applied Pharmacology, Brussels, Belgium;* [2] *Université Catholique de Louvain, Pôle de Pharmacologie et Thérapeutique, Brussels, Belgium;* [3] *Université Catholique de Louvain, Pediatric Pulmonology & Cystic Fibrosis, Brussels, Belgium*

Objectives: Vardenafil, a clinically approved cGMP-dependent phosphodiesterase type 5 inhibitor (PDE5i), is able to increase defective F508del-CFTR chloride transport across the mouse nasal mucosa. This work aimed at studying the effect of vardenafil to rescue CFTR function and localization at the gastrointestinal (GI) tract, affected in 85% of CF patients.
Methods: CFTR function was assessed by quantifying potential difference across the rectal mucosa. Sodium hyperabsorption (40.2±4.0 mV *vs* 20±1.8 mV; $p < 0.001$; mean±SEM) and reduced chloride transport (–4.2±0.5 mV *vs* –9.4±0.9 mV; $p = 0.002$) were typically found in F508del-CFTR (CF) compared to wild-type (WT) mice. Vardenafil, applied as a single intraperitoneal dose (0.14 mg/kg) completely restored chloride transport (–9.3±1.2 mV) in CF. Immunohistostaining studies showed reduced CFTR expression at cell membrane in CF mouse colon preparations: in untreated conditions, the ratio of CFTR-specific fluorescence (RF) of the cell membrane and that of the rest of the cell was lower in CF (1.4±0.1) than in WT (1.9±0.1; $p < 0.001$). The RF was increased in vardenafil-treated (2.2±0.1) compared to untreated CF colon tissues ($p < 0.0001$).
Conclusion: Our findings pointed out the intestinal mucosa as an additional valuable target tissue to study CFTR function and localization and to evaluate efficacy of therapeutic strategies in CF. Vardenafil also restores ion transport abnormalities across the GI epithelium acting as a corrector of the cell mislocalization of F508del-CFTR.

WS7.2 Ex vivo effect of CFTR modulators VX770, VX809 and PTC124 on CFTR-mediated chloride secretion in rectal biopsies from CF patients

S. Scheinert[1], L. Pinders-Kessel[1], N. Derichs[1]. [1] *Charité Universitätsmedizin Berlin, Pediatric Pulmonology and Immunology – CFTR Biomarker Centre/Translational CF Research Group, Berlin, Germany*

Objectives: CFTR modulators VX770 (ivacaftor), VX809 and PTC124 have been investigated in clinical phase trials. Prediction of clinical effects at a preclinical stage is complicated by the lack of optimal models. Aim of this study was to evaluate the preclinical ex vivo effect of CFTR modulators on human rectal CFTR function, to provide a basis for long-term correlations with clinical in vivo effects.
Methods: Rectal suction biopsies (n = 256) from 11 PI-CF patients (6 F508del-homozygous, 5 with nonsense-mutation), 8 PS-CF patients (Class IV-V mutation) and 13 healthy controls were freshly procured for Intestinal Current Measurement (ICM). Transepithelial short-circuit current was measured according to the ECFS ICM SOP, before and after ex vivo incubation for 16 hours (37ºC, 95% O2, 5% CO2) with VX809 (10–30 μM), VX770 (10–30 μM), PTC124 (10 μM), Gentamicin 400 μg/ml, combinations or DMSO 0.1%.
Conclusion: F508del-CFTR function was partially corrected by ex vivo treatment with VX809 to a level up to 25% of non-CF control (DMSO). PTC124 enhanced CFTR function in CF rectal biopsies with at least one nonsense-mutation to 14.4% of non-CF control, whereas gentamicin showed no effect. In PS-CF with class IV-V mutations, VX770 normalised CFTR function from residual to levels of non-CF. These data indicate the high potential of ICM for preclinical CFTR modulator testing in native ex vivo human tissues. The technique might be helpful for mutation-specific individual prediction of clinical in vivo drug effects and early identification of responders and non-responders of CFTR modulating therapies.
This study was supported by Mukoviszidose e.V., Germany.

WS7.3 VX-661, an investigational CFTR corrector, in combination with ivacaftor, a CFTR potentiator, in patients with CF and homozygous for the *F508Del-CFTR* mutation: Interim analysis

S. Donaldson[1], J. Pilewski[2], M. Griese[3], Q. Dong[4], P.-S. Lee[4], for the VX11–661-101 Study Group. [1] *University of North Carolina, Chapel Hill, United States;* [2] *University of Pittsburgh, Pittsburgh, United States;* [3] *University of Munich, Munich, Germany;* [4] *Vertex Pharmaceuticals Incorporated, Cambridge, United States*

The *F508del-CFTR* mutation affects folding and trafficking of CFTR, resulting in defective gating and little to no CFTR activity at the epithelial surface. VX-661, an investigational CFTR corrector, increases F508del-CFTR protein activity in vitro, both alone and in combination with ivacaftor, and appears to enhance F508del-CFTR trafficking to the cell surface. An ongoing study, VX-661-101 (Study 101), evaluates the safety and efficacy of VX-661 alone and in combination with ivacaftor in patients (pts) with cystic fibrosis (CF) homozygous for the *F508del-CFTR* mutation. This interim analysis evaluates the safety of escalating VX-661 doses.
This is a Phase 2, randomized, double-blind, placebo-controlled, 3-part study. In Part A, approximately 120 adult (≥18 yrs) pts homozygous for the *F508del-CFTR* mutation and with FEV_1 40 to 90% of predicted, will be randomized into dosing groups of VX-661 alone (10, 30, 100, or 150 mg qd) or in combination with ivacaftor (150 mg q12 h) for 28 days. The primary measures are safety and change in sweat chloride (baseline–Day 28). Secondary measures include changes in FEV_1 and in CFQ-R (baseline–Day 28).
Approximately 130 pts are expected to enroll into the 10, 30, 100, or 150 mg dosing groups of VX-661 alone or in combination with ivacaftor, and enrollment is expected to be completed by early 2013. Interim analyses results will be reported. Preliminary data, including safety, changes in sweat chloride, FEV_1 % predicted and CFQ-R are anticipated to be available at time of presentation.
The safety and efficacy profile of VX-661, with and without ivacaftor, in adult CF pts homozygous for the *F508del-CFTR* mutation will be reported.

WS7.4 Lumacaftor, an investigational CFTR corrector, in combination with ivacaftor, a CFTR potentiator, in CF patients with the *F508del-CFTR* mutation: Phase 2 interim analysis

M. Boyle[1], S.C. Bell[2], M. Konstan[3], S. Mc Colley[4], P. Flume[5], L. Kang[6], Y. Wu[6], D. Waltz[6], N. Patel[6], for the VX09–809-102 Study Group. [1] *Johns Hopkins University, Baltimore, United States;* [2] *The Prince Charles Hospital, Brisbane, Australia;* [3] *Case Western Reserve University, Cleveland, United States;* [4] *Northwestern University, Chicago, United States;* [5] *Medical University of South Carolina, Charleston, United States;* [6] *Vertex Pharmaceuticals Incorporated, Cambridge, United States*

Lumacaftor enhanced F508del-CFTR protein trafficking in vitro, and addition of ivacaftor to lumacaftor increased chloride transport. The safety/efficacy of lumacaftor alone and in combination with ivacaftor in CF patients (pts) with the *F508del-CFTR* mutation is being evaluated in VX-809 Study 102. This interim analysis evaluates the safety/efficacy of escalating lumacaftor doses.
This is a Phase 2, randomized, placebo-controlled study. In Cohorts 2 (n = 109) and 3 (n = 15), pts received either lumacaftor (Cohort 2: 200, 400 or 600 mg qd; Cohort 3: 400 mg q12 h) for Days 0–28 (Period 1) plus lumacaftor (continued same dose) combined with ivacaftor 250 mg q12 h for Days 28–56 (Period 2), or placebo (Periods 1 and 2). Safety, FEV_1, and sweat chloride were examined.
In Cohort 2, most adverse events were mild/moderate and comparable between treatment groups. A subset of pts reported generally mild and self-limited episodes of dyspnea/chest tightness coincident with initiation of 600 mg qd lumacaftor. Lung function was generally stable in Period 1. Significant increases in FEV_1 were observed during Period 2 in all dose groups vs placebo. The greatest increase in % predicted FEV_1 was observed in the 600 mg lumacaftor plus ivacaftor homozygous group vs placebo (8.6% absolute, 12.8% relative) ($p < 0.001$). In this same group, sweat chloride was reduced with lumacaftor alone (Period 1) (–6.41 mmol/L) and with ivacaftor (Period 2) (additional –3.66 mmol/L) vs placebo.
In this interim analysis of pts with the *F508del-CFTR* mutation, lumacaftor alone or in combination with ivacaftor was well tolerated, and the combination had lung function improvement in Cohort 2. Cohort 3 will be presented.